Densities of Several Proteins and L-Amino Acids in the Dry State

by E. Berlin and M. J. Pallansch

Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C. 20250 *(Received December 19, 1967)*

Densities were determined pycnometrically, by helium displacement, for the anhydrous forms of several proteins, 18 naturally occurring L-amino acids, glycine, and di- and tripeptides of glycine. Values observed for the proteins included 1.261 g/cm³ for β-lactoglobulin and 1.320 g/cm³ for bovine serum albumin. No correlation was observed between the experimentally determined densities of the proteins and the values calculated from their amino acid composition.

Introduction

Early studies by Chick and Martin[1] on the density of proteins in solution or in anhydrous form showed that the proteins underwent a decided contraction upon solution. McMeekin, Groves, and Hipp[2] have studied the density and water content of β-lactoglobulin crystals and showed that the density of the crystals is a linear function of their water content over the range of 14–46% moisture. They reported the values of 0.802 ml/g for the specific volume of anhydrous β-lactoglobulin and 0.772 ml/g for the protein in crystals containing 14–46% water. This difference was attributed to either an apparent packing of the protein due to the high density of the water molecules or to the presence of molecular voids in the crystal which could not be entered by the organic solvent used in measuring the density of the dried crystals.

Haurowitz,[3] in discussing the problems encountered in determining the density of dry-protein or wet-protein preparations, pointed out the difficulties in choosing an appropriate displacing fluid when the density is to be determined pycnometrically. These difficulties include selecting a fluid which enters all voids in the protein crystal and does not interact chemically with the protein.

The technique of gas displacement will, however, circumvent many of these difficulties in density measurements. Helium is usually chosen as the displaced medium, as it is chemically inert, behaves as an ideal gas, does not adsorb on the surface to any appreciable extent at ambient temperatures, and is sufficiently small to enter all voids between as well as within the particles.

We were, therefore, prompted to apply the technique of He displacement to measure the density of anhydrous β-lactoglobulin to determine if any such molecular occlusions could be observed. The availability of

(1) H. Chick and C. J. Martin, *Biochem. J.*, **7**, 92 (1913).

(2) T. L. McMeekin, M. Groves, and N. J. Hipp, *J. Polym. Sci.*, **12**, 309 (1954).

(3) F. Haurowitz, "The Chemistry and Function of Proteins," 2nd ed, Academic Press Inc., New York, N. Y., 1963, pp 113, 114.

different genetic forms of α_s-casein in large quantities also made it possible to compare the densities of these protein forms.

There is a paucity of data in the literature on the densities of the naturally occurring L-amino acids in the dry state. Furthermore, it was of interest to see if any correlations existed between the densities of the component amino acids and the densities of the proteins themselves. It has been demonstrated that calculations of the solution volumes of many proteins from their amino acid compositions give values that are in good agreement with the observed solution volumes, though there are some exceptions.[4]

Experimental Section

β-Lactoglobulin (recrystallized 4 times) was prepared according to the method of Gordon, Semmett, and Ziegler[5] and α-casein (Na salt) was prepared by the method of Hipp, *et al.*[6] The genetic variants of α_s-casein were purified preparations given to us by Dr. M. P. Thompson, Eastern Regional Laboratory, U. S. Department of Agriculture, Philadelphia, Pa. The bovine serum albumin was a preparation of fraction V from bovine plasma purchased from Armour Pharmaceutical Co. All the proteins were dried by lyophilization.

The amino acids and peptides were commercial samples of the highest available purity. Cysteine, glycylglycine, and glycylglycylglycine were purchased from Nutritional Biochemicals Corp. and lysine was purchased from Sigma Chemical Co. All other amino acids were purchased from Calbiochem.

Helium (Southern Oxygen Co.) was purified by passing through a charcoal trap maintained at $-195°$. The charcoal was initially outgassed at 250° for several hours.

Protein densities were measured at 25° by helium displacement using a volumetric gas-adsorption system, as previously described.[7] Prior to measuring the densities, the protein samples were degassed under high vacuum (10^{-6} torr) to constant weight. Most of the water was removed within 16 hr while pumping at ambient temperature; however, the degassing was continued at 40° for 3–5 days. Outgassing was considered complete when there was no mass loss of more than 0.01% of the sample mass in the final 24 hr of drying.

Densities of the amino acids and peptides were measured with a Beckman air-comparison pycnometer using helium as the pycnometric medium. The amino acids were initially dried to constant weight in a vacuum oven at ambient temperature. (Reference to certain products or companies does not imply an endorsement by the department over others not mentioned.)

Results

The density values obtained for the several protein preparations are presented in Table I and the values for the amino acids are presented in Table II. The peptides glycylglycine and glycylglycylglycine exhibited density values of 1.515 and 1.489 g/cm,[3] respectively, when determined by helium displacement.

Table I: Densities of Several Dried Proteins

Protein	Density, g/cm³
Bovine serum albumin	1.320
β-Lactoglobulin	1.261
α_s-A/A-Casein	1.251
α_s-C/C-Casein	1.264
Sodium-α-caseinate	1.616

Table II: Densities of L-Amino Acids

Amino Acid	Density, g/cm³
Arginine	1.325
Alanine	1.371
Aspartic acid	1.636
Cysteine	1.495
Cystine	1.655
Glutamic acid	1.566
Glycine	1.598
Histidine	1.412
Isoleucine	1.201
Leucine	1.167
Lysine	1.237
Methionine	1.311
Phenylalanine	1.315
Proline	1.376
Serine	1.582
Threonine	1.499
Tryptophan	1.303
Tyrosine	1.403
Valine	1.267

Discussion

There is reasonably good agreement between the values reported in this paper for the densities of glycine, L-alanine, L-aspartic acid, L-glutamic acid, L-leucine, L-tyrosine, and L-valine and those listed in the Handbook of Physics and Chemistry. The value 1.376 g/cm³ reported here for the density of L-proline is also in fair agreement with that of 1.35 g/cm³ reported by Wright and Cole.[8]

Values for the density of β-lactoglobulin crystals, as reported in the literature,[9–14] range from 1.146 to 1.50

(4) T. L. McMeekin and K. Marshall, *Science*, **116**, 142 (1952).

(5) W. G. Gordon, W. F. Semmett, and J. Ziegler, *J. Amer. Chem. Soc.*, **76**, 287 (1954).

(6) N. J. Hipp, M. L. Groves, J. H. Custer, and T. L. McMeekin, *J. Dairy Sci.*, **35**, 272 (1952).

(7) E. Berlin and M. J. Pallansch, *ibid.*, **46**, 780 (1963).

(8) B. A. Wright and P. A. Cole, *Acta Crystallogr.*, **2**, 129 (1949).

(9) B. W. Low and F. M. Richards, *J. Amer. Chem. Soc.*, **74**, 1660 (1952).

g/cm³. Many of the variations in density result from differences in water content of the crystals as well as varying amounts of $(NH_4)_2SO_4$ or sucrose occluded within the crystals during the preparation. Water content need not be considered in the present study, since the data reported here are for degassed samples. Furthermore, there should be no problem of molecular voids impenetrable by helium, as discussed by McMeekin, with reference to organic solvents.[2]

The value of 1.261 g/cm³ reported in the present investigation is in excellent agreement with that of 1.260 g/cm³ reported by McMeekin and Warner[12] and that of 1.27 g/cm³ reported by Riley.[14]

Since there was such close agreement between the values for the density of β-lactoglobulin obtained in the present study by He displacement and that obtained by McMeekin and Warner[12] with organic solvents, we may conclude that there were no molecular occlusions penetrable by helium and impenetrable by the organic solvent. Furthermore, when N_2 was used in place of He in the gas-displacement method, no substantial change in density was observed.

The specific volume of a protein is essential for calculating its molecular weight in solution, yet such specific volumes are often difficult to determine experimentally. Cohn and Edsall[15] have, however, described a method for calculating the specific volume of a protein from its amino acid composition, the volume of a protein molecule being the sum of the volumes of its component groups or atoms. Subsequently, McMeekin and Marshall[4] applied this method to calculate the specific volumes of 19 different proteins and found good agreement between the calculated values and those reported in the literature.

In attempting to correlate protein density in the dry state with the densities of the component amino acids, it was necessary to obtain a suitable density value for the amino acid residues as present in the proteins. A correction factor will, therefore, have to be applied to the densities of the amino acids to take account of the water eliminated when the amino acids are bound in the peptide linkage. Cohn and Edsall,[15] using Traube's[16] values for apparent molal volume increments for H and O atoms, subtracted the factor 6.60 cm³ from the observed molal volumes of the amino acids in solution to account for the volume loss of water in forming the peptide bond. The other corrections made by these authors, for changes in covolume and solvent electrostriction when calculating the volume of an amino acid residue in a protein in solution from the volume of a free amino acid in solution, are not applicable to our work in the dry state.

It should be possible to calculate an experimental peptide-bond volume correction, using our data for the densities of glycine and glycylglycine, as the volume of glycylglycine should be equivalent to twice the volume of glycine less the volume of water eliminated in forming a single peptide bond. Such calculations yielded a value of 6.75 cm³, which is close to that of 6.60 cm³ as employed by Cohn and Edsall.[15] Using this factor of 6.75 cm³ for each of the two peptide bonds present in glycylglycylglycine, one may expect a density value of 1.484 g/cm³, which is in excellent agreement with our observed value of 1.489 g/cm.³

When the density of β-lactoglobulin is calculated using the compositional data given by McMeekin[17] or the more recent data of Dawson,[18] values of 1.478 and 1.479 g/cm,³ respectively, are obtained for the density of β-lactoglobulin. These values are substantially higher than the observed value of 1.26 g/cm.³ Furthermore, when the density of the bovine serum albumin was calculated using compositional data obtained in our laboratory for the same sample, a value of 1.492 g/cm³ was obtained, which is also higher than the observed density of 1.320 g/cm.³ Though amino acid composition may vary between different preparations of β-lactoglobulin, it may, nevertheless, be concluded that in the dry state the volume of a protein is not necessarily the sum of the volumes of its component groups. This conclusion is not necessarily in conflict with the results of McMeekin and Marshall,[4] since it does not necessarily follow that the volumes of the constituent amino acids of a protein should be additive in both the dry state and in solution.

Acknowledgment. The authors thank Dr. M. P. Thompson of the Eastern Regional Laboratory, U. S. Department of Agriculture, for providing the samples of α_s-A/A- and α_s-C/C-casein, and B. A. Anderson, E. D. DeVilbiss, and J. J. Salasin for technical assistance.

(10) B. W. Low and F. M. Richards, *J. Amer. Chem. Soc.*, **76**, 2511 (1954).

(11) B. W. Low, F. M. Richards, and J. E. Berger, *ibid.*, **78**, 1107 (1956).

(12) T. L. McMeekin and R. C. Warner, *ibid.*, **64**, 2393 (1942).

(13) T. L. McMeekin, M. L. Groves, and N. J. Hipp, *ibid.*, **72**, 3662 (1950).

(14) D. P. Riley, *Nature*, **168**, 242 (1951).

(15) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, pp 370–381.

(16) J. Traube, *Samml. Chem. Chem.-Tech. Vortrage*, **4**, 255 (1899).

(17) T. L. McMeekin in "The Proteins," Vol. II, Part A, H. Neurath and K. Bailey, Ed., Academic Press Inc., New York, N. Y., 1954, pp 389–434.

(18) R. Dawson, *Biochim. Biophys. Acta*, **49**, 591 (1961).